EFFECT OF PLASMA ANGIOTENSIN CONVERTING ENZYM IN ERECTILE DYSFUNCTION

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ABSTRACT

Objective: *The aim of this study was to know the effect of aging and I/D angiotensin converting enzymes (ACE) gene polymorphisms in modulating plasma ACE level in erectile dysfunction (ED) subjects.* ***Material & method:*** *This case control study carried out in Makassar with 83 male subjects (49 ED subjects and 34 normal subjects). International Index of Erectile Function-5 was used for ED diagnosis while plasma ACE level and genotyping ACE gene examined with ELISA test and Rigat PCR method respectively.* ***Results:*** *The data showed that plasma ACE level siginificantly higher in ED group compared to normal group, while the plasma ACE level consistently raised following the increased of age in ED group but did not statistically significant ($p > 0.05$) compare to normal group. DD genotype in ED group had mean plasma ACE higher compared to ID and II subjects eventhough did not statistically significant.* ***Conclusion:*** *The high level of ACE plasma appeared to have a pivotal rule in ED mechanisms as well as in older ED subjects. DD genotype partly modulated the raising of plasma ACE in ED subject comprared to ID and II genotype.*

Keywords: *Plasma angiotensin converting enzymes level, erectile dysfunction.*

ABSTRAK

Tujuan : *Mengamati pengaruh usia dan polimorfisme I/D gen angiotensin converting enzymes (ACE) pada peninggian kadar ACE plasma yang menyebabkan disfungsi ereksi (DE).* ***Bahan & Cara:*** *Penelitian observasional dengan rancangan case-control ini dilakukan di Makassar dengan melibatkan 83 subyek. Subyek dibagi atas 49 orang kelompok kasus (DE) dan 34 orang kelompok kontrol (non DE) dengan International Index of Erectile Function-5. Pengukuran konsentrasi ACE menggunakan teknik ELISA, sedang genotyping gen ACE dengan PCR menggunakan metode Rigat.* ***Hasil:*** *Didapatkan bahwa kadar ACE plasma lebih tinggi secara signifikan pada kelompok DE ($p < 0.05$), demikian juga bahwa pada kelompok DE kadar ACE plasma makin meningkat sesuai penambahan usia dibandingkan kelompok kontrol meskipun tidak bermakna secara statistik ($p > 0.05$). Genotipe DD gen ACE menyebabkan peningkatan kadar ACE dibanding genotype ID dan II pada kelompok DE meskipun tidak signifikan ($p > 0.05$).* ***Simpulan:*** *Tingginya kadar ACE plasma berperan penting pada kejadian DE. Kejadian DE pada usia yang lebih tua cenderung disebabkan oleh meningkatnya kadar ACE plasma. Nampaknya genotipe DD gen ACE secara parsial menyebabkan meningkatnya kadar ACE plasma yang akhirnya menyebabkan DE.*

Kata kunci: *Kadar angiotensin converting enzymes plasma, disfungsi ereksi.*

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INTRODUCTION

Angiotensin converting enzymes (ACE) is an enzyme that correlates to blood vessel endothelial disorder. Many studies reported the increment of plasma ACE in the cardiovascular diseases, metabolic diseases and erectile dysfunction (ED). ACE is a dipeptidyl carboxypeptidase that plays an important role in converting angiotensin I (ang I) to angiotensin II (ang II) and inactivating bradykinin. The increasing of ang II and decreasing of bradykinin play an important role in endothelial dysfunction disorders.

ED is the inability to achieve and maintain an erection sufficient for satisfactory sexual performance.[1] Yang (2009) also found increased

ACE in cavernosal smooth muscle of ED rats.[2] Some studies described ED based on the increasing level of ACE which will raise the ang II level and inactivate bradykinin.[3,4]

The number of ED patients appeared to be increase from time to time and estimated more than 322 million in 2025.[5] The ED occurrence was reported more common by aging. Chew et al reported 13.1% of 40-49 years have ED and increased to 33.5% in 50-59 years old.[6] Study in animal model revealed the evidence of ultra-structural changes in penile tissue of old rats. But the association of the plasma ACE level and ultrastructural changes in the aging of the ED patients was unclear.

The ACE gene located in chromosome 17q23, consists of 26 exons and 25 introns essential to encode ACE formation. Although many studies reported controversial results, some studies showed the correlation between DD genotype of ACE gene and higher plasma ACE level.

OBJECTIVE

The aim of this study was to know the effect of aging and I/D ACE polymorphisms on increasing ACE plasma that occurred in ED patients.

MATERIAL & METHOD

This case control study involved 83 subjects recruited from outpatient clinic at Wahidin Sudirohusodo General Hospital Makassar and private clinic during 2005. Subjects were divided into two groups (49 ED case group and 43 normal controls). ED was diagnosed using International Index of Erectile Function-5 (IIEF-5), while ACE plasma level and ACE gene genotype were examined from each blood subject.[7] All subjects had been given an informed consent, and the study was approved by the medical ethics committee of Hasanuddin University.

The concentration of plasma ACE was measured by ELISA method (Enzyme Linked Immunoassorbent Assay) by using the ELISA kit (Chemicon International Inc, Temecula, CA 92590). Principle of examination was quantitative sandwich enzyme immunoassay. Specific monoclonal antibody for ACE was already bound in the microplates. Samples and standard were titrated into wells and ACE bind with immobilized antibody. After filtering of unbound substance, specific enzyme-linked polyclonal antibody were added into the well. This solution was filtered a second time to diminish the reagent of unbound enzyme antibody. The substrate solution was added into the well to form color sufficient to bind ACE in initial phase. Staining was stopped then the color intensity was measured at 450 nm speed, using ELISA reader with µg/ml scale. To determine high and low levels, ACE level was sorted into quartiles with the fourth quartile considered high.

Five ml of blood was taken from each subject and DNA was extracted by using standard methods. ACE gene polymorphisms was determined by modified Rigat method of PCR. The PCR primes used in this study were :

GIIS:5 -CTCAAGCACGCCCCTCAACGGACTG-3',

GAS:5 -GATGTGGCCATCACATTCGTCAGAT -3',

FYM :5-ATCACGAGGTCAGGAGATCGAGAC -3'

Amplification of the 16 intron through the insertion area (I) by using primary flanking GIIS and GAS heated for 1,30 minute in 94°C followed by 20 amplification cycle (94°C, 0,30 menit; 62°C, 1 menit; 72°C, 1 menit) within 5 µl buffer containing 2 mM MgCl2 and 0.25% DMSO, using 0.5 U of Goldstar DNA polimeration (Promega USA), and 20 pM primary concentration. The tube then was cooled at 4°C then GIIS and primer FYM was added (each 20 pM/tube) then PCR was continued to 30 cycles under the same conditions, followed by 4 minutes of extra time for extension proccess. Extension between GAS and GIIS produce PCR 561-bp product for insertion (I) allele, and 274-bp product for D allele. Further amplification between FYM and GIIS will produce 376-bp product only for insertion (I) alleles.

Erectile dysfunction was scored by IIEF-5 and defined as ED if the IIEF-5 score was equal to or less than 21.[7]

RESULTS

Table 1 showed sample characteristics and percentage of subjects suffering ED increasing with the age as well as DD genotype of I/D ACE gene. Mean plasma ACE level was significantly higher in ED group compared to normal group.

Table 1. Sample characteristic.

Variable	ED		Normal		p
	n	%	n	%	
Erection function	49	59	34	41	
Age					
40 – 49 year	9	40.9	13	59.1	0.07*
50 – 59 year	21	60.0	14	40.0	
60 – 69 year	19	73.1	7	26.9	
I/D ACE gene					
DD	19	73.1	7	26.9	0.34*
ID	12	57.1	9	42.9	
II	16	55.2	13	44.8	
		Mean ± SD		Mean ± SD	
Plasma ACE level	49	322.2 ± 112.4	34	242.2 ± 109.3	0.002**

p*: chi square test; p**: independent sample t-test .

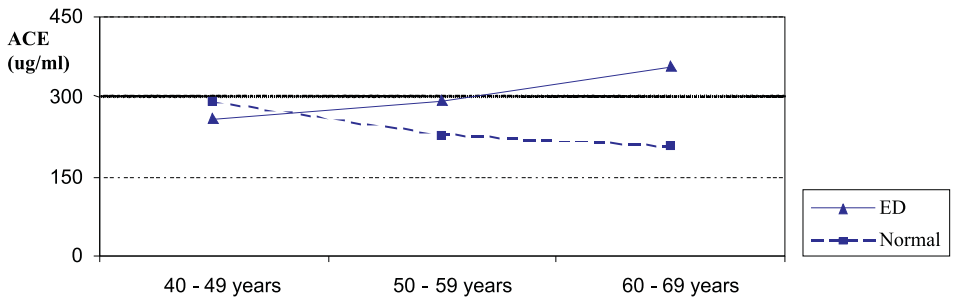

Figure 1. Correlation between age and mean ACE level In ED and normal group

p: Kruskal Wallis test.

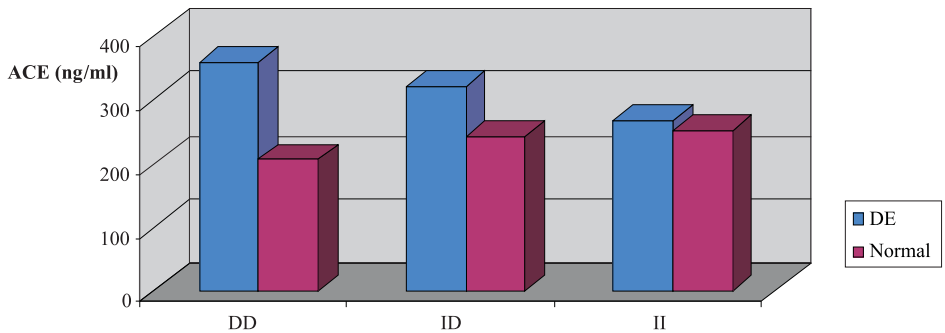

Figure 2. Correlation between ACE gene polymorphism and average ACE plasma level In group with ED and normal group

The level of plasma ACE increased consistently with aging in ED group but not in normal group (figure 1), and DD genotype in ED group had plasma ACE higher than ID and II genotype (figure 2).

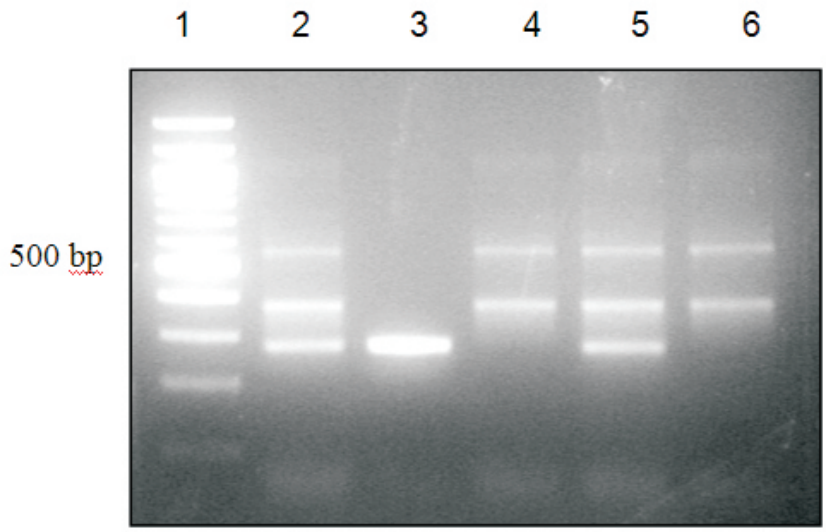

Figure 3. PCR result (1 = Marker DNA, 2 & 5 = ID, 3 = DD and 4 & 6 = II).

DISCUSSION

This study showed high levels of plasma ACE in ED subjects compared to normal subjects (table 1). High ACE level will induced excess plasma ang II, which causes smooth muscle disorder and fibrosis of corpus cavernosum that leads to ED.[8,9] Many studies showed that by reducing the ACE level could improve ED condition. Spell et al (2005), found significant sexual activity improvement by healing corpus cavernosum perfusion using ACE inhibitor for 6 months in ED patients.[10]

Our data also showed the occurrence of ED increasing by age (table 1), as reported by many previous studies.[11,12] Aging process is normally accompanied by many risk factors especially vessel dysfunction. Shen et al (2000) compared penile ultrastructural changes on 9 weeks mice to 62 weeks mice using electron microscope and found tunica albuginea in older mice became thinner, elastic fiber and smooth muscle reduced, and collagen fiber was increased. ACE plasma level tended to increase time to time (figure 1) in ED subject although did not statistically significant ($p > 0,05$).[13] However increasing of plasma ACE in oldest group could make ED getting worse, especially if subject suffered hypertension or diabetic mellitus.

In several studies, DD genotype of ACE gene correlated with hypertension or its complications such as left ventricle hypertrophy (LVH) and chronic kidney disease (CKD), although another study reported a different result. Our study showed that people with ED tend to have DD genotype compared to ID genotype or II genotype but did not statistically significant (table 1). DD genotype on ED group showed the highest ACE plasma mean (figure 2), but in control group DD genotype has the lowest average ACE plasma level compare to ID and II genotype. We assumed that the level of plasma ACE regulated partly by DD genotype and could existed as ED if supported by a bad environment or another risk gene.

CONCLUSION

We concluded that the high level of ACE plasma has an important role in ED occurrence. These enzymes could be modified by age and partly by DD genotype of ACE gene.

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